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A PROGRAM FOR THE STUDY OF SKELETAL MUSCLE CATABOLISM FOLLOWING PHYSICAL TRAUMA

ANNUAL REPORT

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Infusion of PGE_2 into the single hind leg of the dog failed to stimulate the increased release of amino acids. Additional animals underwent lumbar sympathectomy and then sympathetic nerve stimulation. Following sympathectomy, leg blood flow increased, and with stimulation leg blood flow returned toward control values. Glucose Flux did not change in either leg throughout the study and was similar in both hind limbs. Amino acid analysis is presently in process.

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FOREWARD

In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals", prepared by the Committee on the Care and Use of Laboratory Animals in the Institute of Laboratory Animal Resources Commission of Life Sciences, National Research Council (NIH Publication No. 86-23, Revised 1985).

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BACKGROUND

Following injury or infection there is accelerated net breakdown of skeletal muscle protein associated with negative nitrogen balance. A variety of factors influence this response, including food intake, inactivity and the hormonal environment. In studies that control for food intake and exercise, infusion of the catabolic hormones causes negative nitrogen balance but does not account for the accelerated proteolysis that occurs following moderate to severe injury.

The recent recognition that injured tissue or invasive infection stimulates the production of a variety of cytokines has caused a variety of investigators to hypothesize that these mediators may serve as the signal to accelerate skeletal muscle proteolysis. Some of the initial in vitro studies have suggested that increased skeletal muscle protein breakdown may be stimulated by activation of the cyclooxygenase pathway. Others have presented data which demonstrates that proteolysis may continue even when prostinoid mechanisms are not activated.

The initial studies in this contract addressed this issue. They have been covered in previous reports but the data will be reviewed in order to collate the information that has been forwarded in both annual and quarterly reports.

REVIEW OF THE FIRST TWO MAJOR PROTOCOLS

1. The effect of Ibuprofen on skeletal muscle proteolysis

This experiment has been outlined in detail in previous reports. Briefly, 12 dogs underwent laparotomy, retroperitoneal dissection and catheter insertion into the distal aorta and vena cava. They were then studied in the immediate postoperative period, a time which is known to stimulate accelerated protein breakdown. One half of the animals received ibuprofen, in sufficient doses to block cyclooxygenase arachidonate, and the other half served as controls.

The treated animals excreted less urine volume and less urinary nitrogen, although the later value lost significance when the total nitrogen and urea nitrogen were corrected for the animals weight. Measurement of amino acid flux across the hind legs tended to show a reduction in amino acid release at 6 hours post operation, but this difference was not significant. No differences were observed between groups at 24 hours.

We conclude that there is little evidence from this study that cyclooxygenase inhibition effects skeletal muscle protein kinetics in the catabolic phase of injury. If there are effects on ureagenesis, as suggested in this small group of animals studied, they may be mediated by attenuation of the counterregulatory hormones. In particular cortisol, which would diminish hepatic urea generation. ACTH and cortisone attenuation by ibuprofen administration has been clearly shown to occur in humans and dogs, particularly following stimulation with inflammatory mediators such as endotoxin and tumor necrosis factor.

2. The effect of PGE_2 on skeletal muscle proteolysis

The details of this study were outlined last year in the annual report. Briefly, animals had catheters inserted in the femoral arteries of both lower extremities. Six animals served as controls and received saline infusions, and an additional six animals received infusion of PGE2 into one of the extremities, with the other extremity serving as a control. Blood flow and substrate flux was measured across these extremities, to see if PGE_2 could induce activation of the arachidonic acid

cascade and hence induce skeletal muscle proteolysis.

The results demonstrated that PGE_2 increased leg blood flow (from approximately 10 ml/min.kg to 30, $p<0.05$). However, no effect on skeletal muscle proteolysis, as indicated by increase amino acid efflux, was observed. (For specific details, please refer to last years annual report and the December 1988 quarterly report.)

We conclude that PGE_2 infusion does not activate skeletal muscle proteolysis in this model. This data coupled with the result from the first study do not support the hypothesis that prostaglandins play a major role in the mediation of the protein catabolic response to injury.

3. The effect of sympathetic nerve stimulation on skeletal muscle proteolysis

A. Background

It was initially hypothesized that norepinephrine mediated vasoconstriction would stimulate local PGE_2 production. The locally produced prostanoids would then initiate proteolysis. This thesis was tested by stimulation of the sympathetic lumbar chain to one leg of the dog.

B. Animal model

Conditioned mongrel dogs were used for this study. They were fasted over night but allowed access to water and underwent general anesthesia (nembutal) in the early morning. They were intubated, prepped and draped as previously described and in a sterile manner arterial and venous catheters were inserted in the groins bilaterally. The dogs then underwent laparotomy and the sympathetic chain on the right side was dissected free in the lumbar area. The chain was divided at this level so as to prevent reflex changes from occurring following nerve stimulation, and a shielded electrode was placed on the chain between the L5-L6 level, distal to the transection. The abdomen was closed loosely and a carotid artery catheter was inserted in the right neck. A rectal temperature probe was also inserted.

A PAH infusion was started in both of the arterial groin catheters and the animal was allowed to stabilize for at least 1 1/2 hours. At this time (indicated as time 0) blood was drawn simultaneously from the venous catheters in both groins and the arterial catheter in the neck. Triplicate samples were drawn for concentrations of PAH, glucose and amino acids.

Nerve stimulation was then initiated (10 Hz, square wave form with repeated stimulation for 10 seconds every minute) and the stimulations repeated continuously for three hours. The blood samples were repeated at hourly intervals. At the end of the third hour the stimulator was turned off and a recovery sample was obtained one hour later.

Preliminary studies were performed in three animals to determine the type and extent of stimulation that should be utilized. In two animals the stimulation strength was increased from 0-30 Hz over a two hour period. In the third animal stimulation of 10 Hz was used for 2 hours and recovery observed. These preliminary studied allowed us to utilized the protocol as outlined above in six additional animals (see fig 1 for the effects of varying stimulus strength).

C. Results

The six animals studied weighed 22.5 + 1.8 kg. Preparation of the model was carried out without incident and was complete after approximately 2 hours. The animals remained stable throughout the study, although the temperature tended to increase (see Table 1 for vital signs).
Blood flow data from five of the animals behaved in a similar manner. The response to sympathectomy was comparable in the sixth animal but the control leg showed a high calculated blood flow. This animal was considered an outlier and was excluded from the remainder of the analysis.

With sympathectomy, hind limb blood flow increased significantly from 4.5+ 0.6 ml/min.kg to 14.0 +0.6, $p < 0.015$. With nerve stimulation the flow to the sympathectomized limb dropped, but was still greater than that observed in the control extremity (Fig 2). With caseation of the stimulus, leg blood flow remained below postsympathectomy values.

Glucose flux was unchanged in the two limbs at all time points studied (data not shown). Amino acid concentrations are now being determined.

TABLE 1. VITAL SIGNS WITH SYMPATHETIC NERVE STIMULATION TO THE LOWER RIGHT LEG (mean ± S.E.M.)

Time (hr)	Temperature (°c)	Mean blood pressure (mmHg)	Pulse rate (beats/min)	Respirations (/min)
-1	35.7 ± 0.3	135 ± 7	148 ± 9	10 ± 1
0	37.8 ± 0.4	131 ± 6	156 ± 8	13 ± 1
1	38.2 ± 0.2	134 ± 7	157 ± 9	13 ± 2
2	38.5 ± 0.1	130 ± 5	157 ± 4	12 ± 2
3	38.8 ± 0.2	125 ± 5	167 ± 5	14 ± 2
4	39.1 ± 0.2	121 ± 5	172 ± 6	15 ± 2

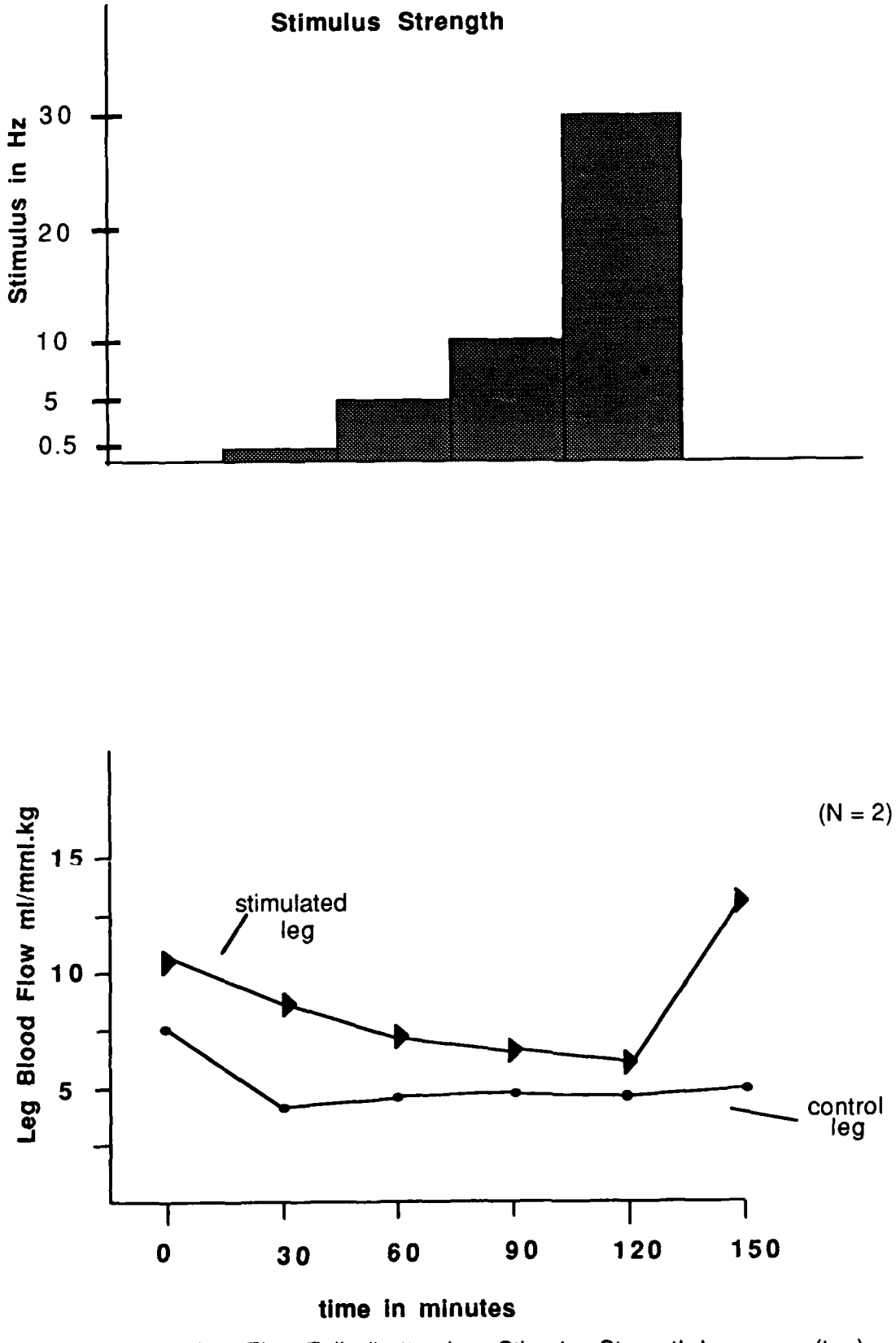

Figure 1. Leg Flow Falls (bottom) as Stimulus Strength Increases (top)

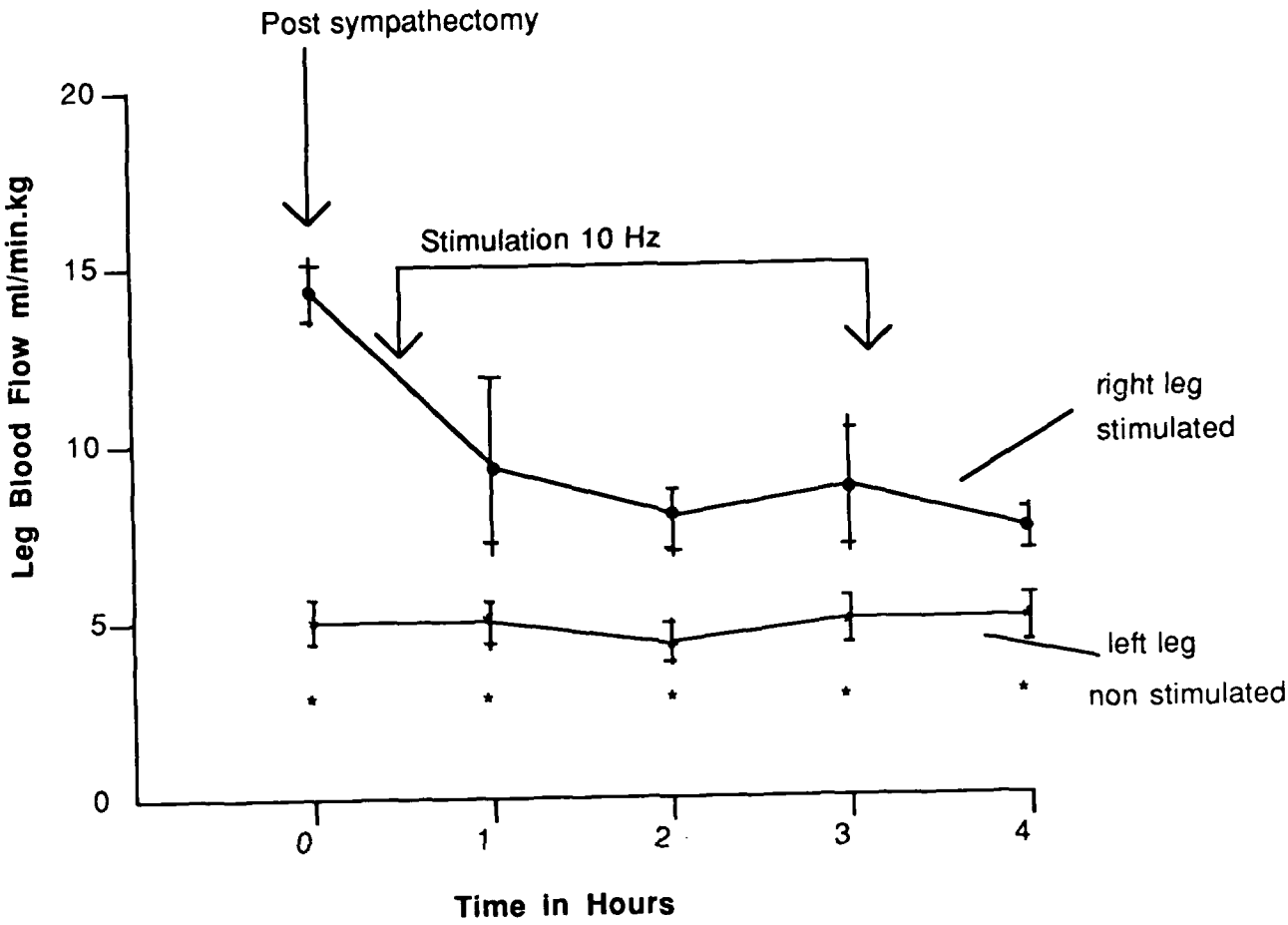

Figure 2. Blood Flow Increases with Sympathectomy, But Falls with Nerve Stimulation. Note the lack of recovery at 4 Hours that occurs with termination of the stimulation.